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Research Article

Identification of Potential Vaccine Candidates from *Rickettsia* Species: A Reverse Vaccinology Approach

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Abstract

Rickettsiosis is a group of diseases caused by many intracellular bacteria of the genus *Rickettsia*. These bacteria are transmitted by ticks, fleas, lice and mites. The public health impact of rickettsiosis has been suspected to be quite high worldwide. However, there is no specific drug or vaccine available for these diseases. Many doctors recommend the antibiotic, doxycycline for this bacterial infection. Many scientists are engaged in vaccine development. In the present study, we applied the approach of reverse vaccinology to identify the potent vaccine candidates which can be considered for most of the species of *Rickettsia*. Six most virulent species of *Rickettsia* viz., *Rickettsia rickettsii*, *Rickettsia conorii*, *Rickettsia australis*, *Rickettsia felis*, *Rickettsia prowazekii* and *Rickettsia typhi* were taken for the study. Total proteomes of all the six species were aligned using BLAST to identify the common proteins among these species. A total 474 common proteins were identified and thereafter each of these common proteins was aligned with human proteome to check homology with human proteins. The BLAST study found total 185 non- homologous proteins out of 474 common proteins. The sub-cellular localization of these 185 non-homologous proteins was predicted using PSORTb and CELLO tools. Besides, transmembrane helices of these non-homologous proteins were also predicted using TMHMM and HMMTOP tools. A total 19 proteins were selected which were predicted to be localized on the membrane having transmembrane helices ≤ 1. These 19 selected proteins were further analyzed for their binding efficiency with major histocompatibilitiy complexes class I and II using BIOMAS and ProPred tools. As a result, a total 10 potential epitopes have been identified as potent vaccine candidates for rickettsiosis.

Introduction

Rickettsiosis is a group of diseases caused by the infection of species of *Rickettsia*. These are obligate intracellular Gram-negative bacilli that represent a diverse collection of bacteria. Human and arthropods are the natural hosts of the genus *Rickettsia*. It has been observed that these bacteria are generally transmitted to humans by the arthropods [1]. The infection in human occurs either from a tick bite or rarely by contamination of cut skin or a wound with faeces of the ticks. The *Rickettsia* species grow within the host cell and secrete hemolysin C and phospholipase D which disturb the phagosomal membrane to avoid defence mechanism through phagocytosis [2]. The diseases, rickettsiosis have been divided into three bio-groups viz., spotted fever group, typhus group and scrub typhus group [3-4]. Spotted fever is a type of tick born disease caused mainly by *Rickettsia rickettsii* present on the skin. There are many sub-types of spotted fever such as Rocky Mountain spotted fever, Mediterranean spotted fever, Queensland tick typhus, Helvetica Spotted fever, Boutonneuse fever. Besides, spotted fever group disease is also caused by several other divergent lineages including *R. conorii*, *R. australis*, *R. mongolotimonae*, *R. slovaca*, *R. honei*, *R. Helvetica*, *R. akari*, *R. japonica*, *R. sibirica* and *R. africae* [5]. The main causative organisms of typhus group are *Rickettsia prowazekii* and *Rickettsia typhi*. The typhus group includes epidemic typhus (also known as louse-borne typhus, classic typhus or sylvatic typhus) and endemic typhus (also known as murine or flea-borne typhus). The most important typhus of this group is epidemic typhus and its common symptoms include fever, chills, headache and other flu-like symptoms [6]. The Scrub typhus is caused by the bacteria, *Rickettsia tsutsugamushi* which has recently been put under the genus *Orientia* due to homology with it and different features compared to other species of *Rickettsia*. Symptoms of scrub typhus include muscle pain, headache, fever, gastrointestinal problems and even hemorrhaging and intravascular coagulation [7].

Clinical diversity of rickettsial infection varies according to the virulence of the *Rickettsia* and host factors such as age, gender, alcoholism and other underlying diseases. All rickettsial infections start with entry into the skin, either through a tick bite or cut or wound abrasions infected by flea or lice. Thereafter, the bacteria enters in the dermal cells, proliferate intra-cellular and ultimately infects the whole body [8].

For treating rickettsial infections, antibiotics of the tetracycline class (most commonly doxycycline) are prescribed which have

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comparatively high degree of efficacy and low toxicity even in children and pregnant women. Depending on the specific pathogen, azithromycin, rifampin, chloramphenicol and fluoroquinolones are also prescribed but these are not universally effective for all rickettsial agents. Besides, these antibiotics have not been evaluated using controlled clinical trials [9]. No vaccine or specific drug is available for preventing rickettsial infections.

The most pathogenic species of *Rickettsia* include *Rickettsia rickettsii*, *Rickettsia conorii*, *Rickettsia australis*, *Rickettsia felis*, *Rickettsia prowazekii* and *Rickettsia typhi*. Use of vaccine against rickettsiosis will prove a good preventive cure since vaccine may confer much strong, long-lasting protective immunity against subsequent re-infection [10]. Instead of culturing the whole organism using conventional methods, reverse vaccinology is used which analyzes the genome and proteome with the help of *in silico* tools to predict various immunogenic antigens. In the present study, we predicted the peptide sequences with the help of reverse vaccinology which can be used as vaccine targets against *Rickettsia* genus.

Materials and Methods

For identification of vaccine candidates for genus *Rickettsia*, proteomes of six species viz., *Rickettsia typhi*, *Rickettsia rickettsii*, *Rickettsia conorii*, *Rickettsia australis*, *Rickettsia felis* and *Rickettsia prowazekii* were selected. The workflow of the approach has been described in figure 1.

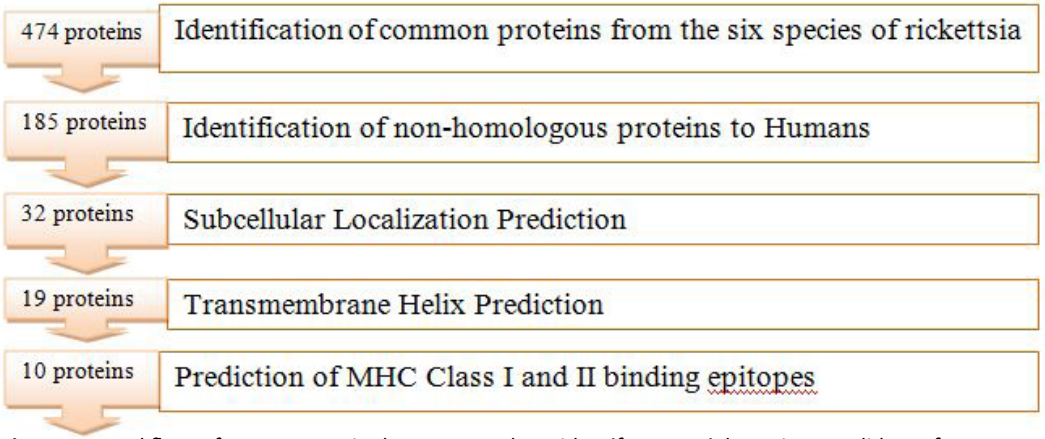

Figure 1: Workflow of reverse vaccinology approach to identify potential vaccine candidates from genus *Rickettsia*.

Retrieval

The whole proteome of *Rickettsia typhi* str. TH1527 (taxid: 1003201), *Rickettsia rickettsii* str. Iowa (taxid: 452659), *Rickettsia conorii* str. Malish 7 (taxid: 272944), *Rickettsia australis* str. Cutlack (taxid: 1105110), *Rickettsia felis* URRWXCal2 (taxid: 315456) and *Rickettsia prowazekii* Rp22 (taxid: 449216) were downloaded from the NCBI genome ftp site ftp://ftp.ncbi.nih.gov/genomes/.

Common Proteins Identification

Homologous similarity search was performed to find out common proteins in all six species of *Rickettsia* using BLAST program [11]. For each sequence, it is necessary to check bidirectional hits which was done by keeping *Rickettsia typhi* protein sequence as reference and thereafter, BLAST was done with *Rickettsia prowazekii* to get similarity. The similar sequences from *R. typhi* and *R. prowazekii* were again BLAST with the other four species by taking bidirectional hits.

Non-Homologous Protein Identification

To prevent auto immune response, identified common proteins were checked for their homology with human using BLAST program. Proteins which were non-homologous were considered further.

Sub Cellular Localization Prediction

For prediction of sub-cellular localization of non-homologous proteins, two different tools viz. PSORTb and CELLO were used [12-13]. Targets localized on membranes of the bacteria, especially on the outer membrane were considered as best vaccine targets since these are exposed to the cell surface and can directly interact with the immune system of the host [14]. The proteins which were either outer membrane bound proteins or extra cellular proteins were considered for further study.

Transmembrane Helix Prediction

Proteins having single transmembrane helix are considered to be better for vaccine designing, as these can be easily isolated from the cells and can be well represented by the endocytic pathway. For better prediction of transmembrane helix, two tools viz., TMHMM and HMMTOP were used [15-16]. Proteins having multiple transmembrane helices were removed from further analysis.

Prediction of Major Histocompatibiltiy Complex (MHC) Class I and II Binding Epitopes

To identify the peptides of all the proteins which bind to MHC class I and II molecules with a good binding affinity, BIMAS and

Protein Name/ID	Selected Peptide	Predicted binding to MHC alleles
YP_005427101.1 zinc/manganese ABC transporter substrate binding protein	LRFFKSLAM FFLNTNIKT LSNNKCIIL	HLA-A1, HLA-A_1101, HLA-0201, HLA-A24, HLA-A_3302, HLA-B60, HLA-B61, HLA-B_3701, HLA-B_5101, DRB1_0102,DRB1_0402, DRB1_0404, DRB1_0405, DRB1_0410,DRB1_0421,DRB1_423, DRB1_0813, DRB1_0408,
YP_005427139.1preprotein translocase subunit SecG	KSTIILTTL ILLIIVILM	HLA-A24,HLA-B14,HLA-60,HLA-B7,HLA-B_2705,HLA-B_3501,HLA-B_3902,HLA- B_5801,HLA- Cw_0602,HLA- Cw_0702,HLA- Ld,DRB1_0101, DRB1_0102, DRB1_0301, DRB1_0305, DRB1_0306, DRB1_0307, DRB1_0308, DRB1_0309, DRB1_0311, DRB1_0401, DRB1_0402, DRB1_0404, DRB1_0405, DRB1_0408, DRB1_0410, DRB1_421, DRB1_0423, DRB1_0426, DRB1_0801, , DRB1_1101, DRB1_1104, DRB1_1106, DRB1_1107, DRB1_1128, , DRB1_1307, DRB1_1311, DRB1_1321, DRB1_1327, DRB1_1328
YP_005427195.1 F0F1 ATP synthase subunit B	IQKLEALRS FYPFFATPS	HLA-A2, HLA-A_0201, HLA-B14, HLA-B8, HLA-B5201, DRB1_0101, DRB1_0102, DRB1_0401, DRB1_0402, DRB1_0404, DRB1_0405, DRB1_0408, DRB1_0410, , DRB1_1101, DRB1_1104, DRB1_1106, DRB1_1121, DRB1_1322, DRB1_1327, DRB1_1328, DRB1_1501, DRB1_1506
YP_005427322.1cell division protein ftsQ	LTILKVLNA	HLA-60,HLA-B7, DRB1_0102, DRB1_0421, DRB1_0423, DRB1_1106, DRB1_1121, DRB1_1128, DRB1_1305, DRB1_1307, DRB1_1311, DRB1_1321, DRB1_1322, DRB1_1323
YP_005427344.1| small heat shock protein	TPLRQVADL VIIMEVPGF	HLA-B14,HLA-B60,HLA-B7,HLA-B_3501,HLA-B_3801,HLA-B_3901, HLA-B_5102, HLA-Cw_0301, HLA-Cw_0401, Cw_0702, Ld, DRB1_0101, DRB1_0102, DRB1_0301, DRB1_0305, DRB1_0306, ALLELE: DRB1_0307, DRB1_0308, DRB1_0309, DRB1_0311, DRB1_0404, DRB1_0405, DRB1_0408, DRB1_0410, DRB1_1307, DRB1_1311, DRB1_1321, DRB1_1322, DRB1_1323, DRB1_1327, DRB1_1328, DRB1_1501, DRB1_1506
YP_005427357.1VirB8-like protein of type	SPVIRYQKL FKYILPLSH	HLA-B60, HLA-B7, HLA-B8, HLA-B_3501, HLA-B_3901, HLA-B_5101, HLA-B_5102, HLA-Cw_0301, HLA-Cw_0301, HLA-Dd, HLA-Kb, HLA-Ld, DRB1_0101, DRB1_0305, DRB1_0401, DRB1_0405, DRB1_0408, DRB1_0426, DRB1_1101, DRB1_1101, DRB1_1104, DRB1_1106, DRB1_1114, DRB1_1128, DRB1_1305, DRB1_1307, DRB1_1311, DRB1_1321, DRB1_1323, DRB5_0101, DRB5_0105
YP_005427456.1 rare lipoprotein A	VLIFCINLS YKVGKNYKI FSGLISILL	HLA-A_0205, HLA-A2, HLa-Cw_0301, HLA-Cw_0401, HLA-B_0702, HLA-A24, DRB1_0306, DRB1_0307, DRB1_0308, DRB1_0402, DRB1_0404, DRB1_0405, DRB1_0423, DRB1_0804, DRB1_0813, DRB1_1101, DRB1_1102, DRB1_1104, DRB1_1106, DRB1_1114, DRB1_1120, DRB1_1121, DRB1_1304, DRB1_1307, DRB1_1328, DRB1_1501, DRB1_1328, DRB1_1506
YP_005427462.1 protocatechuate-3,4-dioxygenase subunit beta	FLYLYTLNI	HLA- B7, HLA-B8, HLA-A3302, HLA-A2, DRB1_0101, DRB1_0102, DRB1_0301, DRB1_0305, DRB1_0306, DRB1_0308, DRB1_0309, DRB1_0311, DRB1_0401, DRB1_0402, DRB1_0404, DRB1_0405, DRB1_0405, DRB1_0408, DRB1_0410, DRB1_0421, DRB1_0423, DRB1_0426, DRB1_0701, DRB1_0703, DRB1_0801, DRB1_1321, DRB1_1322, DRB1_1323, DRB1_1327, DRB1_1328, DRB1_1501, DRB1_1502, DRB1_1506, DRB5_0101, DRB5_0105
YP_005427813.1rod shape-determining protein MreC	VVNSGKLVG	HLA-B3501, HLA-B3801, HLA-A_0205, HLA-A2, HLa-Cw_0301, DRB1_0301, DRB1_0305, DRB1_0306, DRB1_0307, DRB1_0308, DRB1_0309, DRB1_0311, DRB1_0817, DRB1_1101, DRB1_1104, DRB1_1106,DRB1_1107, DRB1_1120, DRB1_1128, DRB1_1311, DRB1_1321, DRB1_1327, DRB1_1328
YP_005427851.1penicillin-binding protein 1A	LKIFAILIL	HLA-B_5101, HLA-B_5801, HLA -A68, HLA- A24, HLA-A3, DRB1_0701, DRB1_0703, DRB1_1101, DRB1_1311, DRB1_1321, DRB1_1322, DRB1_1323, DRB1_1327, DRB1_1328, DRB1_1501, DRB1_1502, DRB1_1506

Table 1: List of peptides predicted to bind to depicted HLA class I and class II molecules.

ProPred tools were used [17-18]. For MHC class I, BIMAS tool was used. In peptide prediction, length of the peptide was fixed to 9-mers, as 9 amino-acid long peptides are best known to bind with the binding sites of MHC I molecules. Peptides binding to all the 41 alleles of Class I HLAs were predicted. For all the alleles only top 10 ranked predicted peptides were considered and rest were ignored. For MHC class II, ProPred tool was used. In this analysis also, the peptide length was fixed to 9-mers. For HLA-II molecules, epitopes for all the 51 alleles of HLA class II molecules were predicted and only top 10 ranked epitopes for each HLA alleles were considered.

Results and Discussion

By using *in silico* approach, 10 proteins have been identified and showed high potential as vaccine targets for the genus *Rickettsia*. A total 474 common proteins were found to be homologous in *R. prowazekii*, *R. typhi*, *R. conorii*, *R. rickettsii*, *R. australis* and *R. felis* using BLAST program. These proteins were considered to be non-homologous to human proteins and a total of 185 non-homologous proteins with respect to human were identified using BLAST. Out of these 185 proteins, 32 proteins were predicted as membrane and extracellular proteins using PSORTb and CELLO tools and were analyzed for the prediction of number of transmembrane helices using TMHMM and HMMTOP tools. The objective of using more than one tool was to reduce chances of failure in experiments. Finally 19 sequences were found to have transmembrane helix (TMH) ≤ 1. Screened transmembrane proteins were used for further analysis of HLA Class I and Class II molecules binding peptides. A total 10 peptides were found to have high efficiency to bind with MHC class I and class II both. The sequences of these 10 peptides are given in Table 1.

Zinc/manganese ABC transporter substrate binding protein (YP_005427101.1) is an extracellular protein that helps the parasite to survive in an environment for its own development. Therefore, it can be a good source of antigen for vaccine development [19]. Preprotein translocase subunit SecG (YP_005427139.1) is a membrane protein and it forms an essential heterotrimeric protein complex which is of prime importance for the Sec pathway. Chawley et al. [20] also reported SecG as good vaccine candidate for *Vibrio cholera*. The F0-F1 ATP synthase subunit B (YP_005427195.1) is an important enzyme which provides energy to the pathogen and is reported as one of the immune-reactive protein [21]. Cell division protein, FtsQ has been reported as target against *Mycobacterium tuberculosis* [22]. Heat shock proteins have been reported as vaccine adjuvant for cancer and infections [23]. The heat shock proteins are the most conserved proteins present in both prokaryotes and eukaryotes [24-25]. However, these heat shock proteins do not have homology in protein sequences. It has been reported that genes coding for prokaryotic and eukaryotic hsp60 and hsp70 have nearly 50% similarity [26-28]. In the present study, comparison of the sequences has been carried out at the protein level where no similarity has been found.

VirB8-like protein has been predicted as an inner membrane protein with one transmembrane helix. The selected epitope sequence lies in the outside region of transmembrane helices that showed that it can interact with the host and is able to generate the immune response. Lipoproteins are immune stimulatory molecules and are the major components of the outer membrane [29]. Lipoproteins are reported as vaccine candidate in *Helicobacter pylori*, *Neisseria meningitides* and *Streptococcus pyogenes* [30-32]. Protocatechuate-3, 4-dioxygenase subunit beta is an enzyme that catalyses the critical ring-opening step in the biodegradation of aromatic compounds. It is a dioxygenase which cleaves molecular oxygen with subsequent incorporation of both oxygen atoms into organic substrates [33]. MreC (murein formation C) protein is involved in the determination of cell shape of the bacteria. Gore et al. reported MreC as a surface antigen in *Listeria monocytogenes* [34]. Penicillin-binding protein 1A is involved in the synthesis of peptidoglycan and is having insensitivity to penicillin [35].

Conclusion

Reverse vaccinology approach used for identification of vaccine candidates for the genus *Rickettsia* is an important approach in narrowing the whole proteome to identify potent vaccine candidates. In the present study, 10 peptides which are common in all the species of *Ricketisia* and are non-homologous to human, and are membrane or extracellular proteins have been predicted as potent vaccine candidates. These identified potent vaccine candidates must be checked using wet lab for the immune response against predicted epitopes.

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